Iron reduction by the cyanobacterium *Synechocystis* sp. PCC 6803

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Abstract
Synechocystis sp. PCC 6803 uptakes iron using a reductive mechanism, similar to that exhibited by many other microalgae. Various bio-electrochemical technologies have made use of this reductive cellular capacity, but there is still a lack of fundamental understanding of cellular reduction rates under different conditions. This study used electrochemical techniques to further investigate the reductive interactions of *Synechocystis* cells with Fe(III) from the iron species potassium ferricyanide, with varying cell and ferricyanide concentrations present. At the lowest cell concentrations tested, cell reduction machinery appeared to kinetically limit the reduction reaction, but ferricyanide reduction rates were mass transport controlled at the higher cell and ferricyanide concentrations studied. Improving the understanding of the reduction of Fe(III) by whole cyanobacterial cells is important for improving the efficiencies of technologies that rely on this interaction.

Key words: Cyanobacteria, iron reduction, reduction rate, electrochemistry, amperometric techniques

1. Introduction

Iron is an essential element for virtually all organisms, but especially for photosynthetic organisms which must maintain their iron-rich photosystems [1, 2]. Due to the low solubility of Fe(III) meaning little iron is bioavailable, several mechanisms for iron uptake have developed. Many algal species such as *Chlorella vulgaris,* along with plants and fungi, are thought to use ferric reductase proteins in their cell membranes to reduce extracellular Fe(III) chelates before internalising them [2-5]. In contrast, iron acquisition by cyanobacteria has been typically considered to be mediated by siderophore binding [6-10]. In *Synechocystis*, genes for siderophore biosynthesis have not been found [7, 11], and the extracellular reduction of Fe(III) species before transport of Fe(II) has been reported [7, 12] consistent with the two Fe(II)/Fe(III) transport systems identified in the plasma membrane; FutABC and FeoB respectively [13-16]. A paper by Kranzler et al. [1] gave the first biological results that demonstrated the reduction of Fe(III) substrates before transport in *Synechocystis*, in line with other microalgae [17, 18]. Bradley et al. [19] gave some

insight into the mechanism of electron transfer, and found that mutant strains of *Synechocystis* sp. PCC 6803 had varying ferricyanide reducing rates depending on the presence of a terminal oxidase complex. However there is still relatively little known about factors affecting iron reduction, and even the effect of light is disputed [1, 20]. McCormick et al. [20] found that wild-type *Synechocystis* 6803 reduced ferricyanide at higher rates in light than in dark, but Kranzler et al. [1] found that iron reduction rates of Fe-EDTA were unaffected by light, and if cells were incubated with a photosynthetic inhibitor it even caused an increase in iron reduction rates.

Understanding the rate of iron reduction is important not only for fundamental understanding at the cellular level, but also for applications that make use of this reductive capacity of whole cells [21, 22]. In a bio-electrochemical cell, electrons harvested from cells by redox mediators such as potassium ferricyanide, $K_3[Fe(CN)_6]$, can be used as a fuel source at an anode for electricity production, or used at a cathode to produce secondary products. This latter approach was adopted in a recent paper by McCormick et al. [20], who utilised *Synechocystis* sp 6803 and its interaction with ferricyanide to produce hydrogen. Various other devices created using *Synechocystis* and ferricyanide to produce electricity have additionally been reported [19, 23]. Although the use of artificial mediators has widely been abandoned in microbial fuel cell operation due to sustainability issues, ferricyanide is still a valuable tool for investigations on metabolic activity. In contrast to many other iron species, the iron atom in ferricyanide is strongly bound and is not released by the ligands after reduction to ferrocyanide, meaning it cannot be internalised by the cell [17]. This makes it highly suitable for probing cell membrane activity for rate studies.

Iron reduction rates are typically quantified colourimetrically [24-26], but can also be measured using liquid scintillation analysis [1, 16], or electrochemically [27, 28]. Electrochemical measurements are carried out by placing an electrode in cell suspensions and measuring the current due to oxidation of any reduced mediator in solution [27, 28]. This was the approach taken in a previous paper by the authors to measure the reduction of ferricyanide by the algal species *C. vulgaris* [29]. In general, electrochemical methods measure the diffusion limited current, i.e. diffusion of the redox mediator between the cell and the electrode is the rate-determining step. As a result, real-time information on enzyme turnover rates is not obtained. However, by using rotating disk electrochemistry (RDE) - a versatile technique where the electrode is rotated at a range of rotation speeds, pulling solution towards the planar disk electrode and resulting in laminar flow across the electrode surface - it is sometimes possible to separate mass transport limited currents from kinetically limited currents by plotting the data according to the Koutecky-Levich equation. A linear response is expected, where the slope contains information on the mass transport limited current and the intercept gives information on any electron transfer limited current. However, in all electrochemical measurements of this type, there will be some error due to trapping of iron at the cell surface [29].

This study uses electrochemical techniques to further investigate the ability of *Synechocystis* sp. PCC 6803 to reduce Fe(III) in ferricyanide with varying concentrations of either ferricyanide or cells present. Ferricyanide reduction was measured electrochemically using both RDE and chronoamperometry at a static macroelectrode. These electrochemical experiments should be able to discriminate between different mechanisms of iron uptake. An oxidation current will only be

measured as the cyanobacteria reduce ferricyanide (Fe(III)) to ferrocyanide (Fe(II)), and release the reduced mediator to the extracellular environment, as outlined in Schematic 1. For species that utilise siderophore complexes to internalise the ferricyanide before reducing it, no oxidation current would be observed. To the best of our knowledge this is the first time that reduction of ferricyanide by intact *Synechocystis* cells has been directly probed by an electrode in this way.

2. Materials and Methods

2.1 Culturing conditions of Synechocystis sp. PCC 6803

Stock (from Crest Lab at the University of Cambridge) was maintained on sterile 2 wt% agar plates made from iron replete BG11 growth media (adjusted to pH 7.5), and grown in iron replete liquid BG11 media under constant agitation (orbital shaker) for experimental use. All cultures were subjected to a 12:12 hour light:dark regime, with fluorescent light supplied from a non-heating diffuse white light source placed under cultures (light box with surface luminal intensity of 1500-1800 cd m^{-2} and light colour temperature of 7000-8000 K from www.lightboxUK.net). Cell counts were performed under an optical microscope using a haemocytometer (Bright-Line from Sigma Aldrich), before cells were prepared for experiments by centrifuging for three minutes at 13.2 x 10^3 rpm, removing the supernatant and re-suspending cells in fresh iron-replete BG11 media at desired concentrations. By utilising the cell growth media itself as an electrolyte during experiments (adjusted to pH 7.5), it was ensured that as far as possible, optimum test conditions were provided for cyanobacterium activity.

2.2 Electrochemistry

All electrochemistry was carried out on an Autolab PGSTAT 12 using a three electrode cell setup (Pt or fluorine doped tin oxide (FTO) working electrode, Pt counter and Ag/AgCl (3 M) reference). To monitor cellular ferricyanide reduction, cells were pipetted into electrolyte solutions containing cell media/ferricyanide, and the working electrode was polarised to 100 mV positive of the potential at which the peak oxidation current (I_p^{ox}) was seen for ferrocyanide. This potential was determined by cyclic voltammetry, and ensured any ferrocyanide generated by the cyanobacteria would be rapidly re-oxidised to ferricyanide at the electrode surface.

RDE was performed using a Pt electrode (area 0.07 cm^2) with limiting currents measured chronoamperometrically at increasing rotation speeds between 100-600 rpm. First, cells were placed in a solution containing 100 % ferricyanide, and the amount of ferrocyanide produced by the cyanobacteria as they reduced the ferricyanide was monitored. The ferricyanide concentration was kept constant at 2.78 mM, before increasing the cell concentration (taking dilutions into account) from 2.40 x 10^7, 4.76 x 10^7, 7.07 x 10^7, 9.34 x 10^7 to 1.15 x 10^8 cells ml^{-1}. Secondly, the cell concentration was held constant, and the ferricyanide concentration increased from 2.78, 5.41, 7.89, 10.26, 12.50, 16.00, 19.23 to 22.22 mM. Finally, the ferricyanide concentration was held constant at 22.22 mM, and cells again increased from 9.26 x 10^7, 1.10 x 10^8, 1.28 x 10^8, 1.45 x 10^8, 1.62 x 10^8 to 1.79 x 10^8 cells ml^{-1}. Measurements were analysed in terms of the Koutecky-Levich equation (Equation 1), where I is the measured current, I_k is the kinetically limited current, F is Faraday's constant, A is the area of the electrode, D is the diffusion coefficient, ω is the rotation speed in rad s^{-1}, ν is the kinematic viscosity, n is the number of electrons transferred in the redox reaction and C is the concentration of ferrocyanide. A linear response is

expected, where the slope of Koutecky-Levich plots ($1/0.62nFAD^{2/3}\nu^{-1/6}$) contains information on the mass transport limited current, and the intercept ($1/I_k$) gives information on any electron transfer-limited current. Each experiment lasted between 3-5 minutes and three repeats were performed using the same culture. Ferrocyanide diffusion coefficients were calculated previously by the authors [29] as 6.8×10^{-6} cm^2 s^{-1} in cell media, decreasing to 5.48×10^{-6} cm^2 s^{-1} with 7.5×10^7 cells ml^{-1} present, and were adjusted for each specific cell concentration in calculations to take into account any missing fraction trapped at the cell surface.

$$\frac{1}{I} = \frac{1}{I_k} + \left(\frac{1}{0.62nFAD^{2/3}\omega^{1/2}\nu^{-1/6}C} \right) \quad (1)$$

Ferricyanide reduction was also studied under static conditions with an FTO working electrode of area 1.33 cm^2. Currents were measured in the dark for a range of cyanobacterial and ferricyanide concentrations in a total volume of 1 ml, and the effect of culture age (14 and 45 days) was investigated. Where used, light was provided by an LED and an optical fibre (620 nm, 5×10^{-3} W cm^{-2}). Three repeats were taken using the same culture, approximately 20 minutes apart, to ensure the system was at equilibrium and the ferricyanide was not damaging the cells in any way, and BG11 media and ferricyanide background controls were subtracted.

3. Results and discussion

When the oxidised mediator ferricyanide was added to a *Synechocystis* sp. PCC 6803 cell suspension, the appearance of the reduced mediator ferrocyanide was immediately detected (and measured as a current) by an electrode polarised at 100 mV positive of the potential where the maximum oxidation current (I_p^{ox}) was seen for ferrocyanide. The specific potential for all measurements was approximately 0.35 V w.r.t Ag/AgCl (3M), and was determined by cyclic voltammetry before experiments were performed; an example is located in supporting information Figure S1. No other electrochemically active redox species were detected by cyclic voltammetry in the potential window of interest - oxygen reduction was not investigated. Figure 1 shows typical currents measured at a static electrode; light induced a small, complex, change in current compared to the relatively large currents in the dark; for traces a) and b) with both cells and ferricyanide present, this amounted to a 10 or 20 % change in current with light onset with regards to the ~2 or ~5 µA current in the dark, respectively. Controls with no cells or ferricyanide present (trace e) or cells and no ferricyanide (trace d), had low currents of ~0 or ~0.1 µA respectively. Although a control with no cells and 1 mM ferricyanide (trace c) had a current of around 1 µA (due to trace levels of ferrocyanide present), these background currents were measured at each ferricyanide concentration used in experiments, and subtracted. The majority of work in this study was performed in the dark to avoid complication, although the effect of light is discussed briefly later.

The reduction of ferricyanide in the dark by *Synechocystis* was measured by RDE, with the amount of produced ferrocyanide studied both as a function of cell and ferricyanide concentration. Examples of current *vs.* time measurements with increasing electrode rotation rate are shown in supporting information Figure S2. Results were then analysed in terms of the Koutecky-Levich equation and plotted

accordingly. In a standard electrochemical measurement, when ferrocyanide is being oxidised at 100 mV positive of the oxidation peak in the absence of cells, the rate of electron transfer at the electrode should be so fast that the intercept of a Koutecky-Levich plot will be zero. I.e. the system is under mass transfer control. However, if the reduction of ferricyanide by the cells (electron transfer at the cell membrane) is the rate determining step, and is slower than both mass transport of the mediator to and from the electrode and electron transfer on the electrode surface, then it is possible that a kinetically limited current due to cell ferricyanide reduction can be extracted from the Koutecky-Levich plot.

Figure 2a shows that at the two lowest cell concentrations a positive intercept was measured in the Koutecky-Levich plots, meaning kinetic limitations applied to the reduction rate. As outlined above, it is unlikely that this kinetic limitation is due to slow oxidation of ferrocyanide at the electrode, as at the applied potential ferrocyanide oxidation is too fast for limitations to be observed. The limitation is also not due to mass transport in the solution as this component of the current has been separated out. Although a non-zero intercept can be seen when the electrode surface is partially blocked due to biofouling, as the intercept was zero at the higher cell concentrations it is highly unlikely that such a mechanism was at work here. We therefore conclude that the rate determining step being measured is most likely to be the reduction of ferricyanide by *Synechocystis*. Other Koutecky-Levich plots at higher cell and ferricyanide concentrations, shown in Figures 2b and 2c, had intercepts of zero – this means that for these conditions, the rate of ferricyanide reduction at the cell membranes was instead mass transport controlled. These latter results are similar to that found with *C. vulgaris,* where mass transport was also determined to be the rate controlling step for ferricyanide reduction [29].

Using the two positive current intercepts in Figure 2a. where kinetic limitations applied to the ferricyanide reduction rate, average reduction rates were calculated (Table 1). In a simple electrochemical system, the ferricyanide reduction rate, ν, can be calculated from the kinetic limiting current using Equation 2. The resulting units of ν are mol cm^{-2} s^{-1}, but the data here has been adjusted to nmol cm^{-2} hr^{-1} per 10^6 cells ml^{-1} to allow for comparison with other rates, both within this study and in the literature.

$$I_K = nFA\nu \qquad 2)$$

Using the slopes of the Koutecky-Levich plots in Figure 2, the total bulk concentration of ferrocyanide in solution was calculated as a function of cell concentration (10^6 cells ml^{-1}). As ferrocyanide is only present in solution if the *Synechocystis* has reduced ferricyanide, the oxidation current is related to the total amount of redox mediator reduced by the cyanobacteria. Figure 3a shows that the total ferrocyanide concentration increased as more ferricyanide was added, suggesting that the rate at which ferricyanide is reduced per cell increases as the amount of available Fe(III) in the environment increases. In contrast, as the cell concentration was increased at a fixed ferricyanide concentration, the total amount of ferrocyanide produced per cell remained relatively constant (Figure 3b). It should, however, be emphasised that when increasing the total number of cells the current also increased; it is just the current per cell that remained constant. Two sets of controls in the presence of media and ferricyanide (1, 3, 5, 7 and 10 mM) were also carried out at

100 mV positive of the ferrocyanide oxidation potential. As expected, these background currents in the absence of cells were small compared to the situation when cells were present, ranging from 0.02 to 0.065 μA with increasing ferricyanide concentration (1 to 10 mM), and showed no detectable increase in oxidation current with increasing rotation speeds. With 1 mM Fe(III) at 600 rpm, the control with no cells present had a current of ~0.02 μA, compared to ~0.25 μA with 1 x 10^8 cells ml^{-1}. See supporting information, Figure S3, for an example.

It appears the cyanobacterial cells reduced ferricyanide at a constant rate, with the mediator reduction rate depending on the mass transport of ferricyanide to the cells, and ferrocyanide away from the cells. Increasing the concentration of ferricyanide increased interactions between cyanobacteria and ferricyanide, thereby rates of reduction and hence bulk ferrocyanide concentration also increased. These trends were also obtained previously with *C. vulgaris* [29].

The interactions of ferricyanide with *Synechocystis* were also investigated at a static electrode in the dark. Previously with *C. vulgaris* at a static electrode, we concluded that as the FTO electrode makes up the bottom of the electrochemical cell, the algae settled on the surface of the electrode bringing high concentrations of trapped ferri/ferrocyanide with them [29]. Background (ferricyanide plus media) subtracted currents for *Synechocystis*, measured under static conditions at a macroelectrode in the dark and taken where stabilised, are shown in Figures 4a and 4b. Figure 4a shows an increase in current due to the oxidation of ferrocyanide at the electrode, (and hence cell ferricyanide reduction rates) as the concentration of ferricyanide in the solution was increased, as also observed with the RDE. However, a marked increase in current per 10^6 cells ml^{-1} was also seen when the cell concentration was increased (Figure 4b). In the RDE experiment, increasing the cell concentration only led to a small increase in ferrocyanide concentration per 10^6 cells ml^{-1}. The most likely explanation for this seemingly conflicting observation is that as with the algae, the cyanobacterial cells settled directly on the electrode surface at the bottom of the electrochemical cell, bringing trapped ferri/ferrocyanide with them. At higher cell concentrations more cells settled on the surface which increases the concentration of ferrocyanide close to the electrode and hence the current. It should be noted that due to the short time-frame of experiments and the fact that no change was observed in measurements over time, it is unlikely that cells were attaching directly to the electrode. Future work could investigate this using a dialysis membrane to cover the electrode.

Figures 4a and 4b also show that the current due to the oxidation of Fe(II) changed depending on the age and growth phase of the S*ynechocystis* culture. With increasing ferricyanide (Figure 4a), oxidation currents increased exponentially with a 14 day old culture, whilst with a 45 day old culture, the current began to plateau. Although the ferricyanide concentrations used for each culture were different, meaning the current value ranges are incomparable, the comparison of different trends is emphasised. With increasing cells and 1 mM ferricyanide (Figure 4b), currents increased in both cases with cell concentration, although currents with the 14 day old culture were approximately five times greater at 1 x 10^8 cells ml^{-1} than the 45 day old culture. When cells were cultured under similar conditions by Monshupanee and Incharoensakdi. [30], stationary growth began around day 24. Therefore, it is probable that the 14 day old culture in this study were in an exponential growth phase,

whereas the 45 day old culture was in a stationary growth phase. Reasons for the difference in the two groups of cell reduction behaviour are not clear, but may correspond to a change in cell activity/physiology with age. Although to the author's knowledge, the effect of culture age has not been investigated with ferri-reductase activity, culture age has been shown to be an important factor with other metabolic cell activities. For example, Krupinska et al. [31] describe large changes in photosynthetic activity throughout the cell cycle, and Mata et al. [32] describe how microalgae are capable of a metabolic shift as a response to changes in various conditions. However, variation in behaviour between the cyanobacteria cell cultures in this study may have also been due to differences in cell viability, not assessed here; future investigations should address this.

Currents from Figure 4b were also normalised in terms of cell concentration, and shown in Figure 4c. When normalised for cell concentration, with increasing cells and constant ferricyanide the ferrocyanide oxidation current increased before decreasing slightly. This pattern is similar to that seen with the rotating disk experiments, where increasing total cells increased overall currents, but when normalised per cell, similar bulk ferricyanide reduction rates were observed. The RDE work showed that under most conditions the ferricyanide reduction rate was likely diffusion limited, but if it is assumed that the system in which cells sit directly on the electrode surface was not diffusion limited, and if the rate determining step is not electrode ferrocyanide oxidation (which is extremely fast at 100 mV positive of the oxidation potential) then it is possible to assume the cell ferricyanide reduction rate as 0.9 nmol Fe(III) cm^{-2} hr^{-1} (10^6 cells ml^{-1})$^{-1}$. This value is calculated from the maximum current of around 0.03 μA.

As this reduction rate is also very similar to the reduction rates measured at low cell concentrations with the rotating disk (in Table 1), it likely that under these conditions, chronoamperometry at the static electrode was in fact additionally measuring kinetically limited currents. As outlined above, it is possible that as the cells settled onto the electrode surface, diffusion distances of ferricyanide to the cells and ferrocyanide to the electrode surface were minimised. If mass transport increased, the rate-limiting step could switch to become ferricyanide reduction by the cyanobacteria. Previously calculated cell ferricyanide reduction rates by the authors for *C. vulgaris* at a static electrode with 1 mM were of the same order of magnitude as that calculated here [29]. Comparing Fe(III) reduction rates with other studies using *Synechocystis* is more difficult due to variation in units and method; in high salt media, McCormick et al. calculated reduction rates in light as ~3 nmol [nmol Chl]$^{-1}$ h^{-1}, and in dark as.~1 nmol [nmol Chl]$^{-1}$ h^{-1} [20], where Chl represents cell chlorophyll. Kranzler et al. calculated Fe reduction rates as around 10 mol Fe $cell^{-1}$ hr^{-1} x 10^{-21} in both dark and red light [1]. The latter unit corresponds to 0.00001 nmol Fe(III) $hr^{-1}10^{-6}$ cells, where Fe corresponds to FeEDTA instead of ferricyanide.

To investigate the effect of photosynthesis on ferricyanide reduction, an optical fibre provided red light, and a resulting change in current could be repeatedly observed over the duration of the ~60 minute experiments (an example is shown in Figure 5). In general, three repeats were performed approximately 20 minutes apart, to allow the current to stabilise in between light and dark periods. Figures 6a and 6b show the calculated approximate photocurrent magnitudes with increasing ferricyanide or cell concentration respectively, where the photocurrent magnitude (δI_L) was calculated as

the current magnitude in the light minus the stable current magnitude in the dark directly before exposure. Interestingly, light seemed to cause either an increase or decrease of the current (positive or negative photocurrent), depending on factors such as ferricyanide concentration (Figure 6a) or culture age (Figure 6b). These results could go some way towards explaining discrepancies between published work on the effect of light on reduction [1, 20]. Both photosynthetic and respiratory chains in cyanobacteria share a quinone/quinol pool [33], allowing for complex interplay between dark and light processes. The complexity of the curve shown in Figure 5 could demonstrate the switching delay of these processes (of around 50 s, as shown by the curve at 1000, 1500 and 2000 s at the dark/light transition).

4. Conclusions

This paper investigated the reduction of Fe(III) in ferricyanide by *Synechocystis* sp. PCC 6803. Although the use of artificial mediators such as ferricyanide is becoming increasingly unfavourable in microbial fuel cell operation due to sustainability issues, it is still a valuable tool for investigations on metabolic activity. In this study, cellular Fe(III) reduction was confirmed by observing ferrocyanide re-oxidation currents in solutions of *Synechocystis* and ferricyanide, at 100 mV positive of the potential at which the peak oxidation current (I_p^{ox}) was seen for ferrocyanide.

At the lowest cell and ferricyanide concentrations used (2.88 mM ferricyanide with both 2.40 x 10^7 and 4.76 x 10^7 cells ml^{-1}), the ferricyanide reduction was shown by rotating disk measurements to be kinetically limited. I.e. electron transfer at the cell membrane was the rate determining step. *Synechocystis* Fe(III) reduction rates in dark were calculated as ~0.9 nmol Fe(III) cm^{-2} hr^{-1} (10^6 cells ml^{-1})$^{-1}$, similar to Fe(III) dark reduction rates calculated previously by the authors for *C. vulgaris*. However, in all other rotating disk measurements with higher cell and ferricyanide concentrations, to maximum concentrations of 22.22 mM ferricyanide and 1.79 x 10^8 cells ml^{-1}, cellular ferricyanide reduction rates were only limited by mass transport. An exception to this possibly existed when a static electrode was used, where cellular ferricyanide reduction rates were calculated close to the kinetically limited rates calculated previously with rotating disk measurements. This may be explained by the fact that as cells settled onto the static (horizontal) electrode, ferricyanide was brought with them, increasing the mass transport of the system. Light induced a ~10-20 % change in current, compared to background currents in the dark. Although these results are only valid for the specified conditions studied here, they are still relevant for anyone using electrochemistry to measure iron reduction by *Synechocystis* sp. PCC 6803. Therefore improving mass transport in a system relying on cell-iron interactions may increase reduction rates; this is an important finding for any resulting device efficiencies.

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Table 1

[Cells] (with 2.88 mM Fe(III)) / cells ml^{-1}	**Reduction rate / nmol Fe(III) cm^{-2} hr^{-1} (10^{6} cells ml^{-1})$^{-1}$**
2.40 x 10^{7}	1.04
4.76 x 10^{7}	0.80

Schematic 1

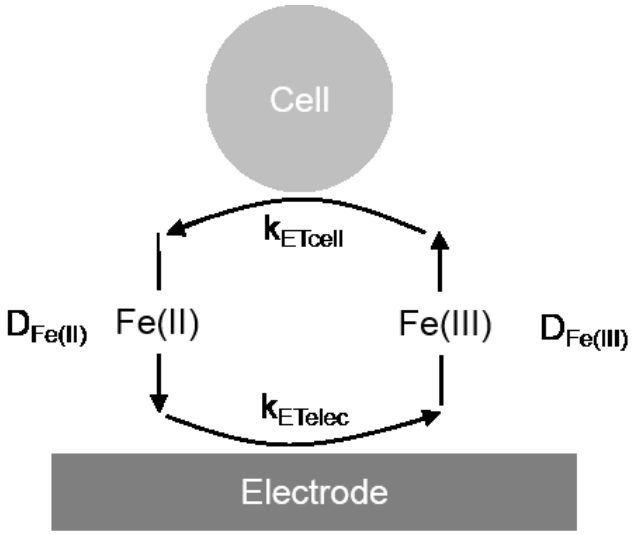

Figure 1

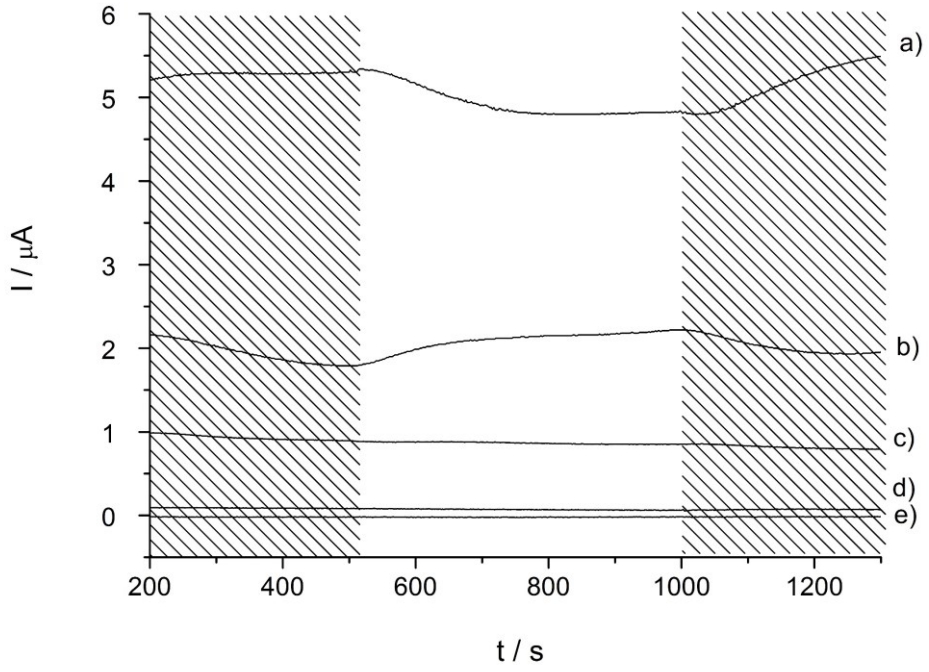

Figure 2

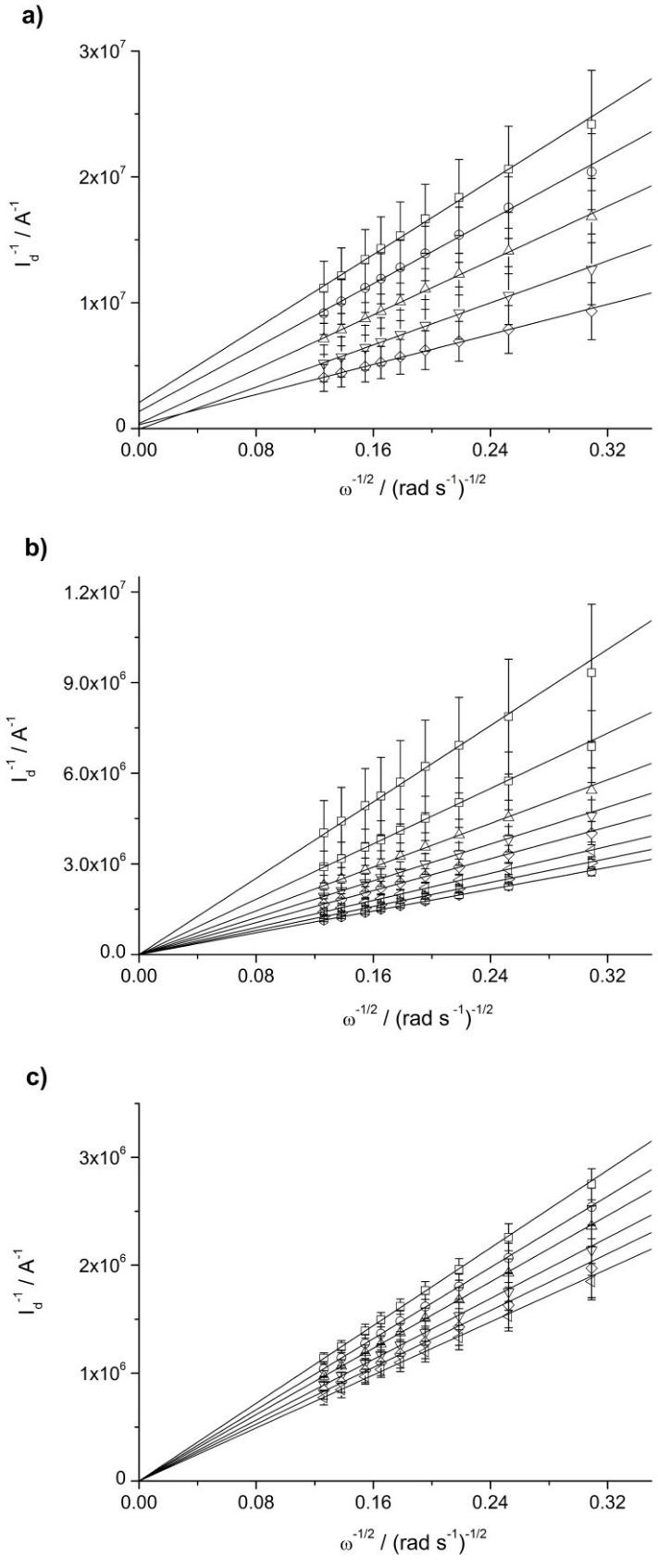
a)
3x10^7
2x10^7
1x10^7
0
I_d^-1 / A^-1
0.00
0.08
0.16
0.24
0.32
ω^-1/2 / (rad s^-1)^-1/2
b)
1.2x10^7
9.0x10^6
6.0x10^6
3.0x10^6
0.0
I_d^-1 / A^-1
0.00
0.08
0.16
0.24
0.32
ω^-1/2 / (rad s^-1)^-1/2
c)
3x10^6
2x10^6
1x10^6
0
I_d^-1 / A^-1
0.00
0.08
0.16
0.24
0.32
ω^-1/2 / (rad s^-1)^-1/2

Figure 3

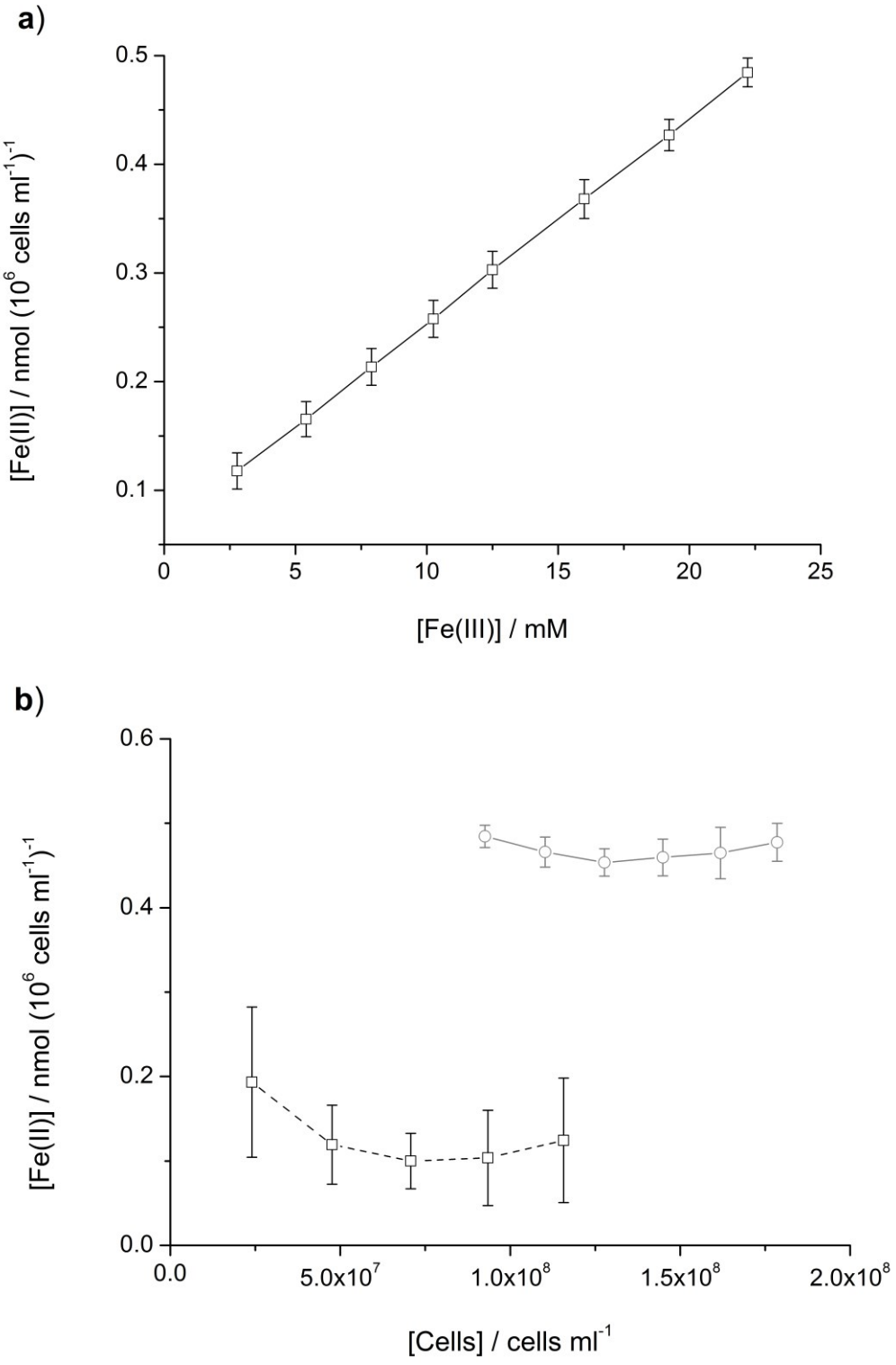
a)
0.5
0.4
0.3
0.2
0.1
[Fe(II)] / nmol (10^6 cells ml^-1)^-1
0
5
10
15
20
25
[Fe(III)] / mM
b)
0.6
0.4
0.2
0.0
[Fe(II)] / nmol (10^6 cells ml^-1)^-1
0.0
5.0x10^7
1.0x10^8
1.5x10^8
2.0x10^8
[Cells] / cells ml^-1

Figure 4

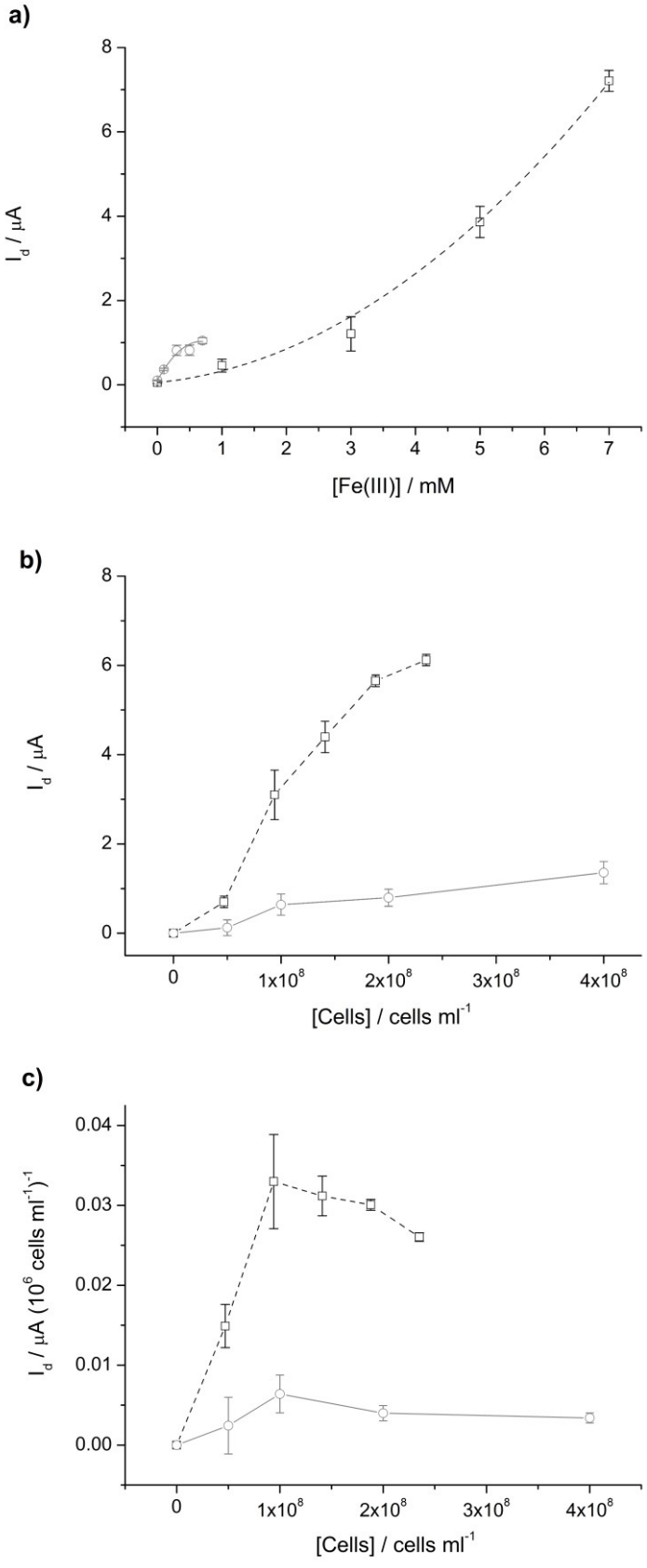
a)
I_d / μA
[Fe(III)] / mM
b)
I_d / μA
[Cells] / cells ml^{-1}
c)
I_d / μA (10^6 cells ml^{-1})$^{-1}$
[Cells] / cells ml^{-1}

Figure 5

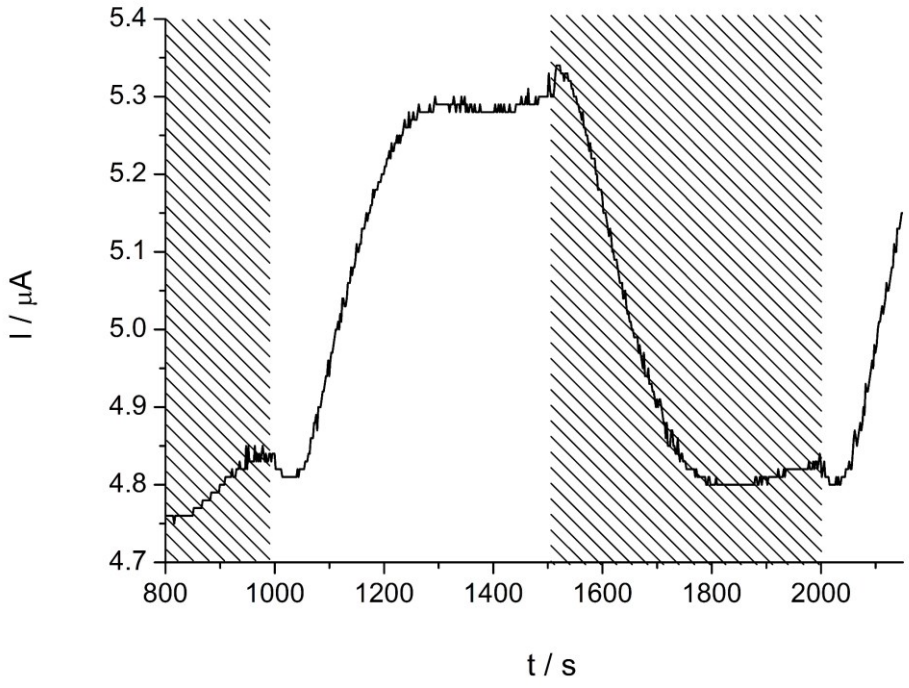

Figure 6

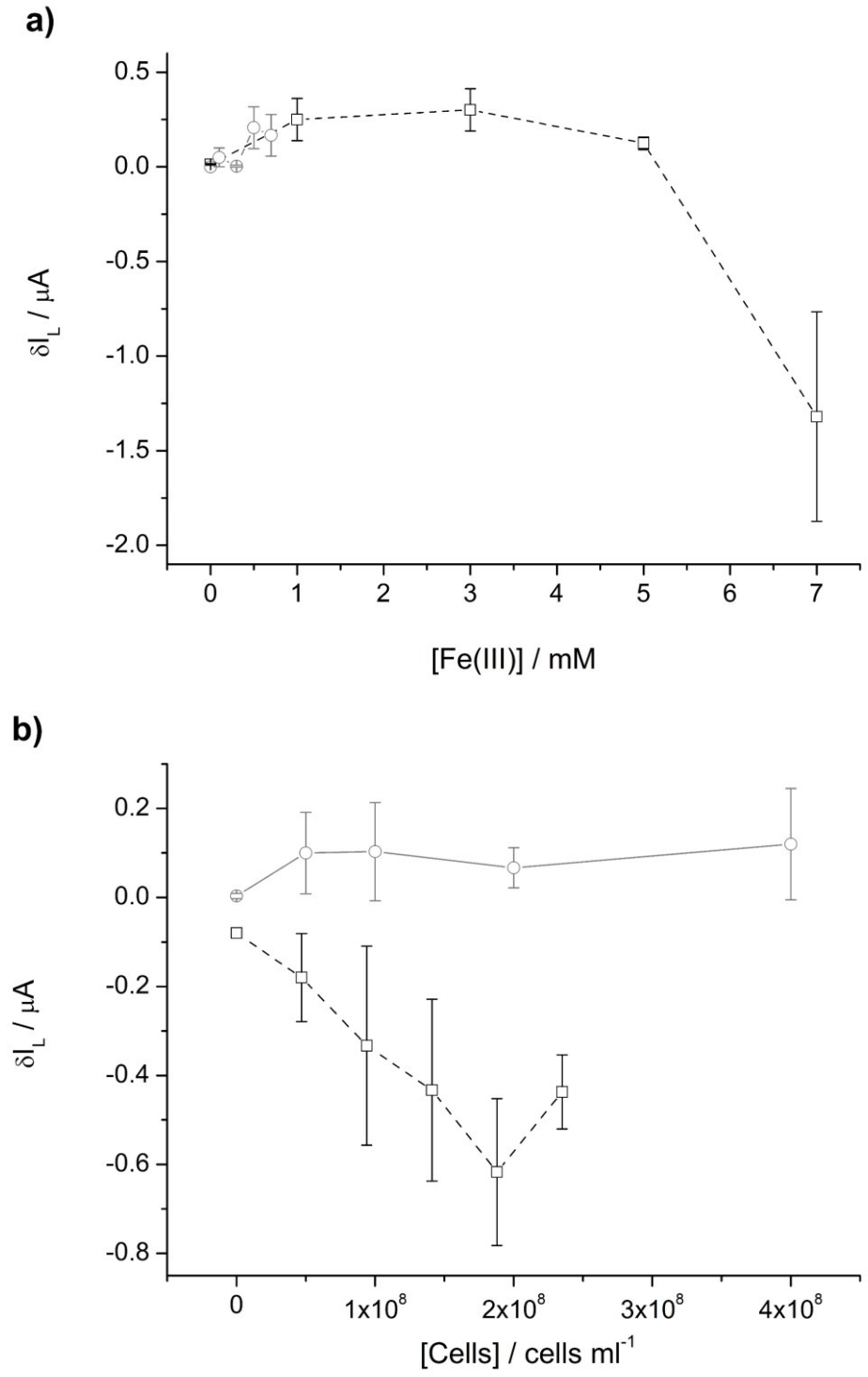
a)
0.5
0.0
-0.5
-1.0
-1.5
-2.0
δI_L / μA
0
1
2
3
4
5
6
7
[Fe(III)] / mM
b)
0.2
0.0
-0.2
-0.4
-0.6
-0.8
δI_L / μA
0
1x10^8
2x10^8
3x10^8
4x10^8
[Cells] / cells ml^-1

Table and Figure Captions

Table 1. Rates of ferricyanide reduction measured from the intercepts of the Koutecky-Levich plots shown in Figure 2a.

Schematic 1. The feedback mechanism used to measure reduction of ferricyanide by *Synechocystis,* where k_{ETcell} and k_{ETelec} are the rates of electron transfer at the cyanobacteria and electrode respectively, and $D_{Fe(II)}$ and $D_{Fe(III)}$ are the diffusion coefficients of ferrocyanide and ferricyanide respectively.

Figure 1. Example currents in dark (shaded areas) and light (non-shaded) measured during chronoamperometry at 100 mV positive of the oxidation potential for ferrocyanide, at a static macroelectrode (FTO, area 1.33 cm^2). a) Negative photocurrent observed with 1.4 x 10^8 cells ml^{-1} (14 day culture) and 1 mM ferricyanide. b) Positive photocurrent observed with 7 x 10^7 cells ml^{-1} (45 day culture) and 3 mM ferricyanide. c) A control with 1 mM ferricyanide and 0 cells ml^{-1}. d) A control with 7 x 10^7 cells ml^{-1} and 0 mM ferricyanide. e) A media control with 0 mM ferricyanide and 0 cells ml^{-1}. Light was provided by an LED and an optical fibre (620 nm, 5 x 10^{-3} W cm^{-2}).

Figure 2. Plots of dark current^{-1} (I_d^{-1}) vs. rotation speed$^{-1/2}$ ($\omega^{-1/2}$). Figure 2a. 2.88 mM ferricyanide and increasing cells in solution. Cell concentrations of plots (from top to bottom) were 2.40 x 10^7, 4.76 x 10^7, 7.08 x 10^7, 9.35 x 10^7 and 1.16 x 10^8 cells ml^{-1} respectively. Figure 2b. 1.16 x 10^8 cells ml^{-1} cells in solution and increasing ferricyanide. Ferricyanide concentrations of plots (from top to bottom) were 2.78, 5.41, 7.89, 10.26, 12.50, 16.00, 19.23 and 22.22 mM respectively. Figure 2c. 22.22 mM ferricyanide and increasing cells in solution. Cell concentrations of plots (from top to bottom) were 9.26 x 10^7, 1.10 x 10^8, 1.28 x 10^8, 1.45 x 10^8, 1.62 x 10^8 and 1.79 x 10^8 cells ml^{-1} respectively. All measurements were at a potential 100 mV positive of the oxidation potential of ferrocyanide. Cultures were 20 days old and the error bars show one standard deviation (n=3).

Figure 3a. Bulk concentration of ferrocyanide ([Fe(II)], related to ferricyanide reduced by cells, per 1 x 10^6 cells ml^{-1}), with increasing ferricyanide ([Fe(III)]) and 1.15 x 10^8 cells ml^{-1}. Figure 3b. Bulk concentration of ferrocyanide ([Fe(II)], per 10^6 cells ml^{-1}), with increasing cells and either 2.88 mM (black squares, dash line) or 22.22 mM (grey circles, solid line) ferricyanide present. Error bars show one standard deviation (n=3).

Figure 4. Background subtracted dark current (I_d) measured during chronoamperometry at a FTO electrode (1.33 cm^2 set to 100 mV past the oxidation potential for ferrocyanide); with either a varying concentration of ferricyanide and 7 x 10^7 cells ml^{-1} in a total volume of 1 ml (Figure 4a), or with 1 mM ferricyanide and varying concentrations of cells (Figure 4b). Figure 4c shows data from Figure 4b normalised in terms of cell concentration. Cultures of two ages, 14 day old (black squares, dash line) and 45 day old (grey circles, solid line), were used for comparison. Error bars show one standard deviation (n=3).

Figure 5. Positive photocurrent observed with 7 x 10^7 cells ml^{-1} (14 day culture) and 3 mM ferricyanide, measured during chronoamperometry at 100 mV positive of the

oxidation potential for ferrocyanide, at a static macroelectrode (FTO, area 1.33 cm^2). Light was provided by an LED and an optical fibre (620 nm, 5 x 10^{-3} W cm^{-2}).

Figure 6. Photocurrent magnitudes (δI_L) measured as a direct response to light during chronoamperometry at a FTO electrode (1.3 cm^2 polarised to 100 mV positive of the oxidation potential for ferrocyanide); with either a varying concentration of ferricyanide and 7 x 10^7 cells ml^{-1} in a total volume of 1 ml (Figure 6a), or with 1 mM ferricyanide and varying concentrations of cells (Figure 6b). Cells of two ages – 14 day old (black squares, dash line) and 45 day old (grey circles, solid line) were used for comparison. Error bars show one standard deviation (n=3).